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THE RELATIONSHIP OF RADIATION DOSE TO LETHALITY AMONG EXERCISED ANIMALS EXPOSED TO ROENTGEN RAYS

D. J. Kimeldorf and D. C. Jones

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Interim Report

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ABSTRACT

The mortality of exercised irradiated rats was compared with that of non-exercised irradiated rats following x-ray doses ranging from 200 to 1000 r. Swimming to exhaustion under standard conditions of testing constituted the exercise.

Where exposure to x-rays is followed by daily exhaustive exercise the mortality is significantly greater than with irradiation alone. The calculated median lethal dose of x-rays for non-exercised animals is 28 percent higher than that calculated for exercised animals. Several deaths occurred among exercised animals at a radiation dose which was not lethal for non-exercised animals. The mortality is not affected by exercise undertaken prior to irradiation. Experiments demonstrated the fact that the specific environment associated with the method of exercise is not responsible for the mortality increase observed with exercise.

THE RELATIONSHIP OF RADIATION DOSE TO LETHALITY AMONG EXERCISED ANIMALS EXPOSED TO ROENTGEN RAYS*

D. J. Kimeldorf and D. C. Jones

INTRODUCTION

The irradiated mammal appears to be unusually sensitive to a variety of experimental conditions which are tolerated by the non-irradiated animal. Prolonged exposure to cold,[1] daily exhaustive exercise,[2] exposure to normally non-pathogenic organisms,[3] and controlled trauma[4] are examples of diverse treatment to which irradiated animals are considerably more sensitive than non-irradiated control animals. In addition, these conditions are capable of altering the mortality resulting from exposure to radiation: when irradiated animals are subjected to these conditions the result is a greater number of deaths than is observed following irradiation alone.

The purpose of the present study is to assess the extent to which exhaustive exercise influences the mortality of rats irradiated with various doses of x-rays. Mortality data were obtained on exercised rats exposed to a dose range of x-rays found to be sub-lethal to absolutely lethal for simultaneously irradiated non-exercised control rats. Several additional correlative experiments were carried out in order to analyze the relationships between the conditions of exercise and the altered mortality response observed for exercised irradiated animals.

MATERIALS AND METHODS

Male rats of the Sprague-Dawley strain, bred in the laboratory colony and maintained in air-conditioned quarters under optimum conditions, were used in this study. Animals of comparable age and weight were selected to determine effects at the various x-ray dose levels. All animals were weighed daily for two weeks prior to experimentation and individuals not having normal growth increments were discarded. Of the remainder, only those within ± eight percent of the mean weight were accepted. The animals in each weight class were from litters born the same week. Food and water were supplied ad lib. except that food was withdrawn two hours prior to each swimming trial. In all studies the animals were caged individually.

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The Exercise Performance Test. The exercised animals performed a standardized exhaustive exercise test developed in this Laboratory for rats. In brief, they were swum individually in 24-gal. cylindrical metal tanks filled with fresh water to a constant level. These tanks were thoroughly cleaned daily and were refilled prior to each day's trials. The water temperature was adjusted to a given temperature $\pm$ 1.5°C within the range of 15° to 21° C. A lead weight was clipped to the chest fur during each swimming trial. Test rats swam until they sank and could no longer rise above a line approximately 18 in. below the surface of the water. The animal was then retrieved and the duration of the swimming trial was recorded. Exhaustive exercise trials were held daily five times per week for two weeks prior to irradiation and for four weeks following irradiation. The pre-irradiation exercise trials were designed to accommodate animals to the test conditions of exercise. After preliminary conditioning for two weeks most animals could swim 15-30 minutes before becoming exhausted under the test conditions.

Irradiation Procedures. The radiation factors for most of the experiments were 250 KVP x-rays; 15 ma; 0.5 mm Cu + 1.0 mm Al (HVL 1.5 mm Cu); 40 in. TSD; 25 r per min. (air dose) measured with Victoreen thimble chambers. In a few studies the dose rate was altered to 8.5 r per min. with the amperage changed to 8 ma. For exposure to the x-ray beam, animals were placed in lucite chambers spaced radially on a motor-driven turntable. The turntable was revolved slowly in the radiation field. Twenty animals were placed in the beam at each exposure.

In most instances the data reported are the cumulative survival data for two or more experiments at each dose of radiation. Only experiments in which irradiated control and experimental animals were under the beam simultaneously are reported. Data were combined from matched experiments since it was felt that repeated studies would increase the reliability of the experimental data. Post-irradiation exercise was initiated on the day of irradiation, which is considered day one in survival data.

RESULTS

In the study of the dose--mortality relationships, 326 non-exercised and 319 exercised irradiated male rats were used. The mortality data resulting from these studies are summarized in Figure 1 and Table 1.

Over the dose range studied, the mortality for exercised animals was greater per roentgen than for non-exercised animals up to the region of the absolute lethal dose (1000 r). The median lethal dose for exercised irradiated animals was significantly lower than that for non-exercised animals. The calculated median lethal dose for non-exercised irradiated animals was 710 r with a probability of 19/20 that the true MLD for these data is

between 667 r and 757 r. For exercised irradiated animals the MLD was 510 r with the same probability that the true median lethal dose is between 479 r and 544 r. At the median lethal dose, irradiation followed by daily exhaustive exercise was approximately 1.4 times more potent than irradiation without exercise in the mortality produced. Exhaustive exercise in the absence of radiation was ineffective as a cause of death among the more than 500 non-irradiated exercised animals. Deaths occurred with 400 r when irradiated animals were exercised, while all non-exercised irradiated animals survived this dose. Apparently, daily exhaustive exercise is a stress sufficient to cause deaths when concurrent with a dose of radiation ordinarily not lethal for irradiated animals.

The post-irradiation survival time of exercised animals was altered

The observed survival for the period of testing is tabulated for purposes of comparison in Table 3. Probability of a difference as great as that observed between the irradiated controls and experimental groups occurring by chance is indicated. These probability values were obtained by the corrected chi-square formula method.

Exposure to water at either temperature did not alter the mortality of irradiated animals significantly from that of the non-exposed irradiated control animals. The survival rate for irradiated animals exposed to 15°C water did not differ from that of animals exposed to 21°C water ($p>0.50$). Neither the nature nor the degree of an exposure stress was detectable by body weight changes or by clinical observations of the water-exposed animals. The survival of irradiated animals exercised in water of either temperature was significantly lower than that of non-exercised irradiated controls ($p<0.01$), and the mortality rates did not differ significantly between the irradiated animals exercised at the two temperatures ($p>0.10$). It appears, then, that the irradiation mortality is relatively independent of the water temperature (over the range tested) but is dependent upon whether the animals were or were not exercised following irradiation. In addition, merely exposing the irradiated rats to water after irradiation has no significant effect upon mortality.

An additional study was undertaken to determine if exposure to water could alter the length of the post-irradiation survival period. A highly lethal radiation dose (860 r) was used in order to provide the experiment with large numbers of animals dying during the observation period. Approximately 190 animals were split into groups as designated in Table 4. The duration and conditions of exposure have been previously described. Animals were exposed to water daily five days per week for two weeks prior to irradiation; after irradiation they were exposed daily five times per week until the last experimental animal had died. The effective time for 50 percent mortality was calculated by Litchfield's probit method.[6] Data for a series of exercised animals irradiated with 860 r are included for purposes of comparison in Table 4.

The median survival times were nearly identical for all water-exposed irradiated groups. It is concluded that exposure to water does not, by itself, significantly alter the post-irradiation survival time after highly lethal amounts of radiation.

Animals used in the principal studies were accommodated to the swimming tests by daily exhaustive exercise five times per week for two weeks prior to irradiation. The influence of this pre-irradiation exercise upon the mortality during the post-irradiation period was studied in 168 animals. These were segregated into groups as tested and summarized in Table 5.

It is apparent in this experiment that the survival of irradiated animals is significantly altered by daily exhaustive exercise after irradiation, but not by exercise prior to radiation exposure. Accommodation to the test by exercise prior to irradiation did not markedly influence the mortality of animals exercised after irradiation ($p > 0.20$).

DISCUSSION

As measured by the resulting mortality, the irradiated animal is highly sensitive to the stress of daily exhaustive exercise in the swimming performance test. The extent of this stress is somewhat indicated by the occurrence of deaths (23 percent) among exercised animals following an amount of radiation (400 r) which is non-lethal for normal animals. In order to alter the mortality, it is deduced that exercise must occur after irradiation, since exercise prior to irradiation was shown to not significantly influence mortality. Since the probit curves for mortality of exercised and non-exercised animals are parallel, within the experimental error, it appears that the influence of exercise upon mortality is a function of the mortality level which occurs with non-exercised animals in the dose range studied. This suggests that the mechanism of mortality is similar for exercised and non-exercised animals.

Swimming to exhaustion was selected as the technique of exercise in these studies since it represents an intensive form of muscular effort during high motivation, presumably with survival as the goal. This method has been used in several studies of the physiological state associated with intense muscular activity[7,8,9] and in studies evaluating the capacity to do work.[10-13] It is recognized that the swimming procedure necessitates exposure of animals to an unusual environment for a brief period daily. In view of the mortality data obtained from the studies of water exposure, it is concluded that exposure to the conditions of testing is not responsible for the mortality observed with swum-irradiated animals.

Smith and Smith[14] were unable to alter significantly the mortality rate of irradiated mice by subjecting them to forced exercise on a treadmill. Under the conditions of the experiment, the mice were exercised for a specified duration of time, although not necessarily to exhaustion. From the data of Steinhaus and his collaborators[15,16] it appears that the intensity of exercise occurring during swimming performance is greater than that obtained by treadmill techniques in terms of physiological response. In view of the results obtained by Smith and Smith, it may be that intensity of exercise and degree of exhaustion are the significant factors altering the mortality of irradiated animals by exercise. This assumes that there are no species differences involved. These factors are being varied independently in performance studies now being pursued at this Laboratory.

SUMMARY

If animals are exercised by swimming to exhaustion daily after irradiation, there results a greater mortality, with more deaths at lower doses, than with irradiation alone. The calculated median lethal dose of x-rays for non-exercised animals is 28 percent higher than that calculated for exercised animals. Exercise effectively alters the level of mortality when undertaken after irradiation, whereas exercise prior to irradiation has no significant effect upon mortality.

Approved by: M C Fishler
Head, Biological and
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For the Scientific Director

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TABLE 1

Analysis of mortality data by the probit methods of Litchfield and Wilcoxon.[5]

Experimental Conditions	No. of Animals	LD_{50} (r)	Confidence Limits LD_{50} (p = 0.95)	Slope Function	Confidence Limits of Slope Function (p = 0.95)
Non-exercised irradiated (200-1000 r)	326	710	667-757	1.23	1.17-1.29
Exercised irradiated (200-1000 r)	319	510	479-544	1.29	1.24-1.33
Experimental condition tested	**SR/f_{SR}***		**Confidence limits SR (p = 0.95)**	**PR/f_{PR}****	**Confidence limits PR (p = 0.95)**
Exercise	1.05/1.07		0.98-1.13	1.39/1.08	1.29-1.50

* If SR (slope ratio) is less than f_{SR}, then the experimental condition is comparable with the control condition within experimental error.

** If PR (potency ratio) exceeds f_{PR}, then there is a significant shift in the degree of lethality attributable to exercise.

TABLE 2

Distribution of deaths for animals dying during the 30 days of observation. The cumulative mortality is indicated for seven day intervals.

Dose	Conditions	Number of Animals	First Death (Days PI*)	Percent Dead				Median Time Survival (Days PI*)
				Days 1-7	Days 8-14	Days 15-21	Days 22-28	
200 r	Non-Exercised	20	-		None died			---
	Exercised	19	-		None died			---
300 r	Non-Exercised	20	-		None died			---
	Exercised	16	-		None died			---
400 r	Non-Exercised	60	-	0	0	0	0	---
	Exercised	43	13	0	7	12	23	18
500 r	Non-Exercised	84	8	0	5	5	5	12
	Exercised	77	5	5	17	36	40	14
600 r	Non-Exercised	40	6	8	22	25	28	9
	Exercised	60	8	0	23	52	68	15
700 r	Non-Exercised	38	8	0	21	44	44	13
	Exercised	36	3	14	81	92	92	10
860 r	Non-Exercised	40	5	20	88	88	88	9
	Exercised	43	2	84	98	100	100	4
1000 r	Non-Exercised	24	4	100	100	100	100	4
	Exercised	25	3	100	100	100	100	4

* Days post-irradiation.

TABLE 3

Percent survival for irradiated animals (500 r) following daily treatment as noted for two weeks prior to irradiation, and for four weeks post-irradiation.

Experimental Condition	No. of Animals	No. Alive	Percent Alive	p
Irradiated controls	65	60	92	
Exposed to 15°C water	25	22	88	>0.80
Exposed to 21°C water	25	24	94	>0.80
Exercised in 15°C water	42	30	71	<0.01
Exercised in 21°C water	54	29	54	<0.01
Non-irrad. exposed (15°C)	25	25	100	>0.30
Non-irrad. exercised (15°C)	25	25	100	>0.30

TABLE 4

The median survival time (ET-50%) for groups of irradiated animals (860 r) exposed to water but not exercised. The ET-50% was calculated by the probit method of Litchfield.[6]

Experimental Conditions	Animals	Mortality	ET-50% (Days)	Confidence Interval (p = 0.95)
Irradiated controls	37	100	9.0	8.6-9.4
Exposed prior to irradiation	39	100	9.5	8.8-10.3
Exposed after irradiation	37	100	9.3	8.9-9.7
Exposed before and after irradiation	39	100	8.6	8.1-9.1
Exercised before and after irradiation	43	100	4.3	3.8-4.8
Exposed but not irradiated	33	0	---	-----

TABLE 5

Percent survival for irradiated animals (500 r) following daily exercise for two weeks prior to irradiation and/or four weeks post-irradiation.

Experimental Conditions	No. of Animals	No. Alive	Percent Alive	p
Irradiated only	40	39	97	
Exercised prior to irradiation only	35	33	94	>0.80
Exercised after irradiation only	31	24	77	<0.05
Exercised before and after irradiation	62	40	64	<0.01

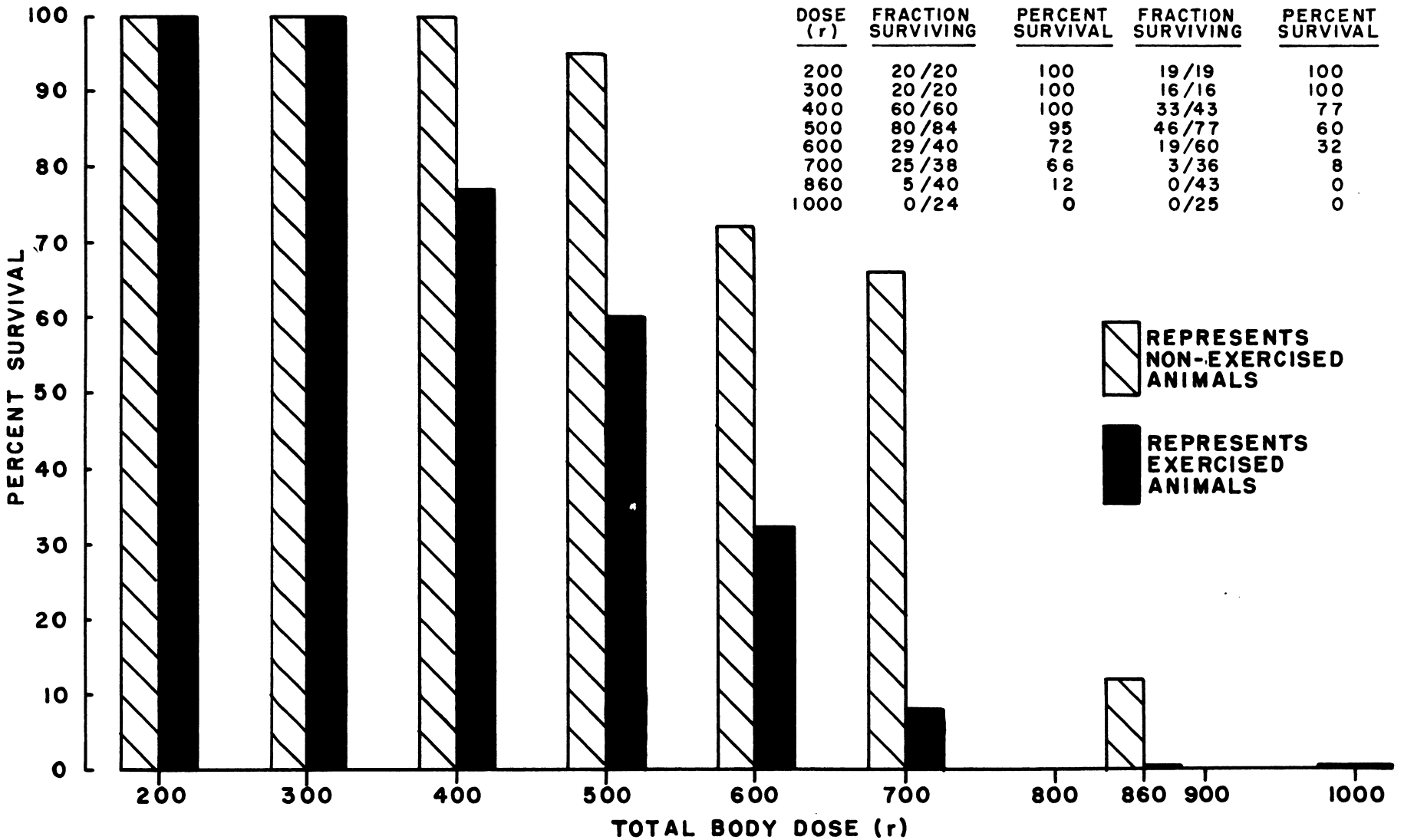

FIG. 1 Percent survival of exercised and non-exercised irradiated rats at 30 days post-irradiation (X-ray).

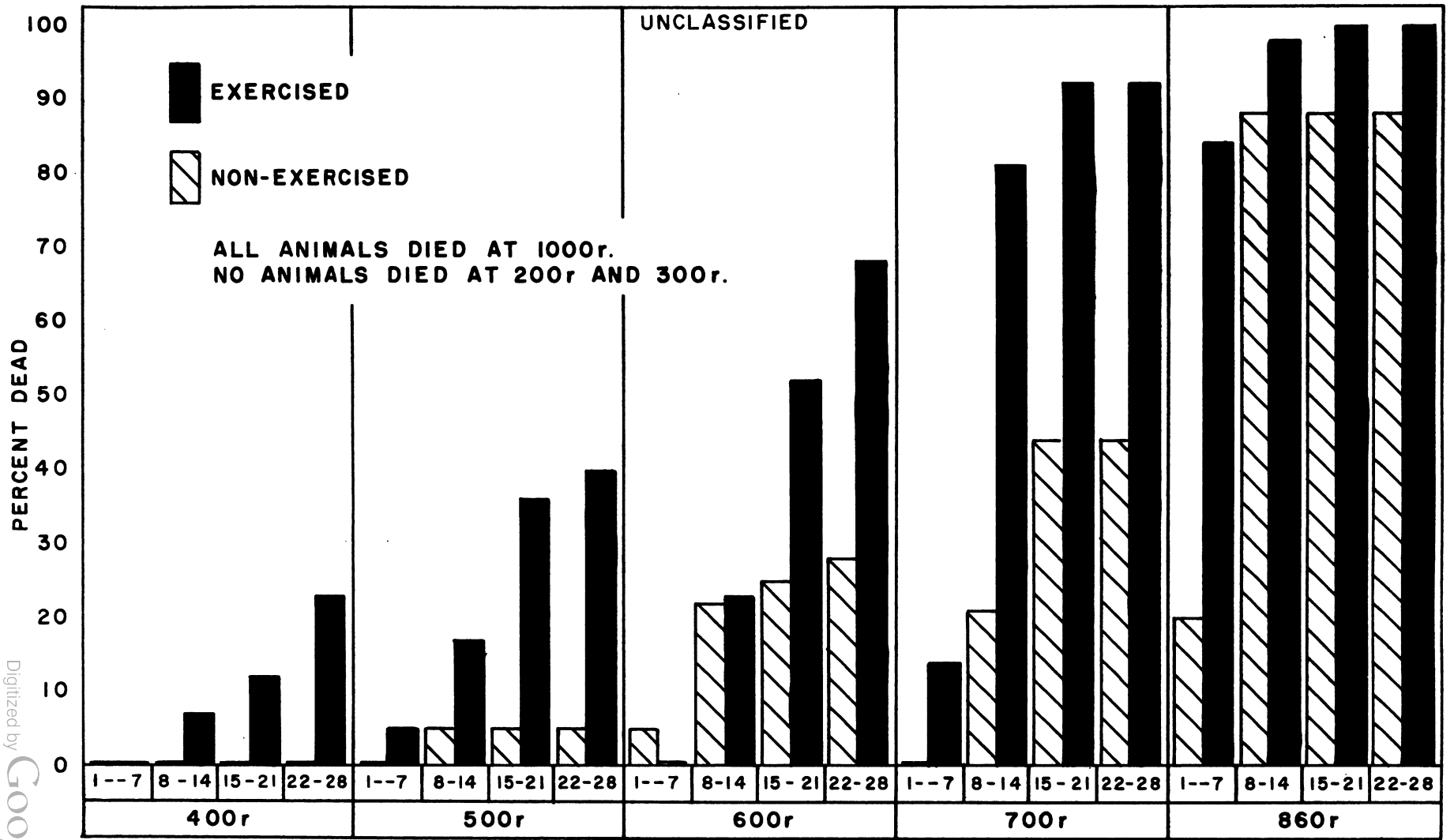

FIG. 2 Distribution of deaths for exercised and non-exercised irradiated rats during 30 days of observation. Data expressed as the accumulative percent dead for each seven day interval post-irradiation.